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Analysis of the factors predicting clinical response to tocilizumab therapy in patients with severe COVID-19

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- ***Highlights.***

  - Improvement at 14 days occurs in 63.3% of severe COVID-19 treated with tocilizumab.
  - Early administration of tocilizumab is associated with better clinical response.
  - A recruitment window of 48 hours from admission in tocilizumab RCTs is advisable.

Complete title: Analysis of the factors predicting clinical response to tocilizumab therapy in patients with severe COVID-19.

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Abstract

Background: Controversy remains about the efficacy of tocilizumab (TCZ) for the treatment of severe COVID-19. We aimed to analyze the profile of TCZ-respondent patients.

Methods: We retrospectively analyzed a cohort of patients with severe COVID-19 admitted to the University Hospital "12 de Octubre" until May, 2020 that received off-label TCZ after indication of a local Committee. The primary end point was significant clinical improvement at day 14 after TCZ (SCI). Factors independently related with SCI were analyzed by multivariate logistic regression models.

Results: Out of 428 patients treated with TCZ, 271(63.3%) experienced SCI. After adjustment by factors related with unfavorable outcome, TCZ administration within the first 48 hours from admission (odds ratio [OR]: 1.98, 95% confidence Interval [95% CI]: 1.1 – 3.55; P-value = 0.02) and ALT levels >100 UI/L at day 0 (OR: 3.28; 95% CI:1.3 –8.1; P-value = 0.01) were independently related with SCI. The rate of SCI significantly decreased according timing of TCZ: 70.2% first 48 hours from admission, 58.5% day 3 to 7 and 45.1% beyond day 7 (P-values = 0.03 and 0.001 respectively).

Conclusion: TCZ improves the prognosis of patients with COVID-19 mostly if treatment is started within the first 48 hours of admission.

- ***Highlights.***
  - Improvement at 14 days occurs in 63.3% of severe COVID-19 treated with tocilizumab.
  - Early administration of tocilizumab is associated with better clinical

response.

- A recruitment window of 48 hours from admission in tocilizumab RCTs is advisable.

List of abbreviations.

ALT: alanine transaminase

ARDS: acute respiratory distress syndrome

AST: aspartate transaminase

COVID-19: coronavirus disease 2019

CRP C-reactive protein

ePO_2/FiO_2: estimated arterial oxygen/fraction of inspired oxygen ratio

HCQ: hydroxychloroquine

ICU: intensive care unit

IFN-β: interferon-β

IQR: interquartile range

IL-6: interleukin-6

IMV: invasive mechanical ventilation

LDH: lactate dehydrogenase

LPV/r: lopinavir/ritonavir

NAT: nucleic acid testing

OTR: oxygen therapy requirements

SCI: Significant clinical improvement

RT-PCR: reverse transcription polymerase chain reaction

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

TCZ: tocilizumab

Introduction

Since the early beginning of the pandemics, the deleterious impact of the hyperactive immune response triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was reported (Giamarellos-Bourboulis et al., 2020, Vabret et al., 2020) and therapeutic immunomodulation emerged as a potentially life-saving option for patients with severe coronavirus disease 2019 (COVID-19) (Luis et al., 2021). Available drugs inhibiting the pleiotropic pro-inflammatory cytokine IL-6 rapidly turned of particular interest, since elevated IL-6 levels seemed to mediate systemic inflammatory responses associated to SARS-CoV-2 infection and the development of acute respiratory distress syndrome (ARDS) and multiorgan failure (McGonagle et al., 2020). Preliminary case series and cohort studies reported outcomes in patients with severe COVID-19 pneumonia treated off-label with intravenous (IV) or subcutaneous (SC) tocilizumab (TCZ), the humanized monoclonal antibody targeting the IL-6 receptor (IL-6R) most available at that time (Antwi-Amoabeng et al., 2020, Fernandez-Ruiz et al., 2021b, Jordan et al., 2020, Knorr et al., 2020, Toniati et al., 2020), with preliminary data suggesting the safety and potential efficacy of this approach. These early results rapidly prompted the incorporation of this agent in most COVID-19 management protocols pending the results of observational studies and randomized clinical trials (RCTs) investigating the real role of TCZ for this indication.

Contradictory results were firstly obtained from observational studies. Although most of these studies found benefits in form of decreased mortality or invasive mechanical ventilation (IMV) requirements among TCZ-treated patients (Deftereos et al., 2020, Mikulska et al., 2020, Rojas-Marte et al., 2020, Rossotti

et al., 2020, Roumier et al., 2021, Somers et al., 2020), some others failed to demonstrate significant outcome differences compared to the standard of care (Campochiaro et al., 2020, Della-Torre et al., 2020). Surprisingly, similar variable results are currently being reported from RCTs. Although recent meta-analyses including all the 10 RCTs with available results up to May 2021 (Snow et al., 2021, Tleyjeh et al., 2021) found an overall statistically significant but modest benefit in terms of mortality in the most severe patients and a trend for lower risk of progression to IMV, the specific results from placebo-controlled RCTs failed to demonstrate a significant prognostic effect (Rosas et al., 2021, Stone et al., 2020). In these regard, there is a need to identify specific factors that would prompt tocilizumab use and some experts are currently claiming for the identification of the clinical profile of the patients most likely to be respondents to TCZ therapy in order to optimize the results of this potentially effective treatment for severe COVID-19 (Fernandez-Ruiz et al., 2021a, Klopfenstein et al., 2021).

The aim of the present study was to analyze the baseline clinical factors related with clinical response in a broad homogeneous cohort of patients that received off-label treatment with TCZ under an institutional protocol throughout the first COVID-19 pandemic wave.

Materials and Methods

Study population and design

This retrospective study was conducted at the University Hospital "12 de Octubre" (Spain). The research was performed in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

The study population included all patients ≥18 years consecutively admitted at our center from March 16 to May 16, 2020 that received IV TCZ as immunomodulatory therapy for COVID-19 pneumonia.

Demographics and major comorbidities; symptoms at presentation; vital signs, laboratory values and radiological features at day 0; use of antiviral therapy; evolution of clinical status at days +1, +3, +5, +7, +10 and +14; treatment-related adverse events; and outcomes were collected from electronic medical records using a standardized case report form. Day 0 was defined as the calendar date in which the first dose of TCZ was administered. Participants were followed-up for 28 days or death (whichever occurred first).

The primary outcome was significant clinical improvement (SCI), defined by hospital discharge and/or a decrease of ≥2 points from baseline (day 0) on the six-point ordinal scale detailed below, by day +14 after the first TCZ dose.

The National Early Warning Score (NEWS) was calculated at admission. Respiratory function was assessed by the pulse oximetry oxygen saturation/fraction of inspired oxygen (SpO_2/FiO_2) ratio. Dynamic changes in the clinical status were assessed according to the following six-point ordinal scale: 1.- discharged to home; 2.- admitted to the hospital, not requiring supplemental oxygen; 3.- admitted to the hospital, requiring low-flow supplemental oxygen

(FiO_2 <40%); 4.- admitted to the hospital, requiring high-flow supplemental oxygen (FiO_2 ≥40%) or non-invasive mechanical ventilation; 5.- admitted to the hospital, requiring IMV, extracorporeal membrane oxygenation (ECMO), or both; and 6.- death. Comorbidity burden was assessed by means of the age-adjusted Charlson comorbidity index. Immunosuppression was defined by the presence of solid organ transplantation, human immunodeficiency virus infection, or receipt of corticosteroid therapy (prednisone ≥20 mg daily or equivalent dose for more than one week), cytostatic agents or other immunosuppressive drugs within the previous month.

Antiviral and immunomodulatory therapies

According to clinical guidelines issued by the Spanish Ministry of Health during the study period (Ministerio de Sanidad, 2020), off-label antiviral regimens included coformulated lopinavir/ritonavir (LPV/r) (200/100 mg twice daily for up to 14 days), hydroxychloroquine (HCQ) (400 mg twice for the first day, followed by 200 mg twice daily for 5-10 days), and subcutaneous (SC) interferon (IFN)-β (250 μg every 48 hours). In addition, some patients received IV remdesivir (200 mg during the first day, followed by 100 mg daily for 5 to 10 days) in the context of an ongoing RCT. Corticosteroid were administered at different dosing regimens (IV methylprednisolone 0.5-1 mg/Kg daily for ≤5 days or as pulses of 100 to 250 mg for 3 days). By April 2020, the use of corticosteroids was generalized for patients presenting to the Emergency Room with COVID-19 pneumonia and SpO2 <92% on room air, regardless of the subsequent administration of TCZ. Most patients received empirical antibiotic therapy (usually with a second or third generation cephalosporin) and thromboprophylaxis with low-molecular-weight heparin (SC enoxaparin 40 mg

once daily, 60 mg once daily, or 40 mg twice daily if body weight <80 Kg, 80 to 100 Kg, or >100 Kg, respectively, with renal dose adjustment if needed).

Beginning March 18, a local Multidisciplinary Committee that included representatives from different clinical specialties and from the Department of Pharmacy was established to standardize decisions regarding immunomodulatory therapies for COVID-19 patients. The committee held daily meetings (except for the weekends) during the first pandemic wave. The off-label use of TCZ was considered for patients potentially eligible for intensive care unit (ICU) admission, with bilateral or rapidly progressive infiltrates in chest X-ray or computerized tomography (CT) scan, and fulfilling one or more of the following criteria: (a) respiratory rate >30 bpm and/or pulse oximetry oxygen saturation (SpO_2) <92% while breathing room air; (b) C-reactive protein (CRP) level >10 mg/dL; (c) IL-6 level >40 pg/mL; and/or (d) D-dimers >1,500 ng/mL. Exclusion criteria included liver function abnormalities (alanine transaminase [ALT] and/or aspartate transaminase [AST] levels >5 times the upper limit of normal), uncontrolled bacterial or fungal infection, or acute diverticulitis or bowel perforation. An initial IV 400 mg (if body weight <75 Kg) or 600 mg (if body weight ≥75 Kg) dose was administered as one-hour infusion. Until March 26, a second 400 mg dose was routinely administered 12 hours later, whereas a third dose could be given after 24 hours from the first infusion for selected patients according to the criteria of the treating physician (Ministerio de Sanidad, 2020). After that date, a single dose was prescribed according to the updated recommendations of the Ministry of Health of Spain.

Statistical analysis

Quantitative data were shown as the mean and standard deviation (SD) or the

median with interquartile range (IQR), whereas qualitative variables were expressed as absolute and relative frequencies. Categorical variables were compared using the χ^2 test. Student's t-test or Mann-Whitney U test were applied for continuous variables, as appropriate. No imputation for missing data was applied.

A multivariate logistic regression model was created to analyze factors independently related with SCI by day +14 on the basis of clinical factors and laboratory values available at baseline (day 0). Those variables with univariate *P*-values ≤0.1 were entered into a backward stepwise logistic regression model. Some continuous variables of interest were dichotomized according to the optimal cut-off value, as determined by the Youden's index. Goodness-of-fit was assessed by the Hosmer-Lemeshow test. Multicollinearity among explanatory variables was analyzed using the variance inflation factor (VIF), with VIF values <3 being considered acceptable. The most parsimonious model (i.e. the highest outcome variability explained with the lowest number of variables) was selected. Results are given as odds ratios (ORs) with 95% confidence intervals (CIs).

All the significance tests were two-tailed. The threshold for significance was set at a *P*-value <0.05. Statistical analysis was performed with SPSS version 20.0 (IBM Corp., Armonk, NY) and graphs were generated with Prism version 6.0 (GraphPad Software Inc., La Jolla, CA).

Results

Clinical characteristics of study groups

After excluding 7 patients with insufficient follow-up data due to transfer to another institution, a total of 428 patients were included in the analysis. As shown in **Table 1** and **Table 2**, most patients were male, of Caucasian ethnicity, with a mean age of 55 years, more than half with some underlying disease and presented to the Emergency Department with cough, dyspnea and diffuse infiltrates on the initial chest X-ray after a median interval of 7 days since symptom onset. Most patients were treated with HCQ, half of them received corticosteroid therapy, and about one third received LPV/r prior to or simultaneously with the first dose of TCZ. Empiric antibiotic therapy including second- or third-generation cephalosporins or amoxicillin/clavulanic acid was also common.

Clinical and analytical data of patients at the time of TCZ administration are depicted in **Table 3**. According to the clinical criteria for being considered as candidates for TCZ by the Multidisciplinary Committee, one single criteria was fulfilled in 13 (3.0%) of them, two criteria in 80 (18.7%), three in 207 (48.4%), four in 116 (27.1%) and five in 12 (2.8%). TCZ was administered mainly at single dose schedule at a median of two days from hospital admission.

As shown in **Table 4**, in the entire study cohort, 271 out of 428 patients experienced SCI by day +14, accounting for an overall rate of 63.3% (95% CI: 58.6 – 67.9). Regarding other outcome parameters, ICU admission was required in 98 patients (22.9%), IMV in 93 (21.7%) and 30-day all-cause mortality was 13.8% (59/428). Reported adverse events after TCZ treatment were bacterial superinfection in 13 patients (3%) and hypertransaminasemia in

30 (7%). No cases of disseminated strongyloidiasis or other opportunistic infections were reported in our cohort.

Factors predicting clinical response by day +14

The preliminary comparative analysis between patients presenting or not SCI by day +14 is shown in **Table 5**. The mean time to TCZ administration from admission was significantly lower in patients that experienced SCI by day +14 (2.9 days vs. 4.9 days; *P*-value = <0.0001). Area under the receiver operating characteristics curve analyses supported by the Youden's index yielded the cut-off of 48 hours from hospital admission as the most predictive with regards to the achievement of SCI by day +14. As shown in **Figure 1**, the rate of SCI by day +14 was significantly higher in those patients receiving TCZ within the first 48 hours (165/235 [70.2%]), compared with 58.5% (83/142) for those treated between days 3 and 7, and 45.1% (23/51) for those receiving TCZ beyond day 7 (*P*-values = 0.03 and 0.001, respectively). Median serum ALT levels at day 0 were also significantly higher in the group with SCI compared to those without (43 vs. 36 IU/L, respectively; *P*-value = 0.001), and the cut-off of 100 IU/L was selected as the most predictive in term of combined sensitivity and specificity.

Conversely, increased age, certain comorbidities (hypertension, dyslipidemia, obesity, chronic obstructive pulmonary disease, immunosuppression and solid malignancy), active or former smoking habit and clinical and analytical data indicative of severe disease at day 0 (low SpO_2/FiO_2 ratio, high leukocyte and low lymphocyte counts, high leukocyte-to-lymphocyte, high CRP, LDH and ferritin levels, bilateral alveolar infiltrates and prior or concomitant corticosteroid therapy) were found to be significantly more frequent in patients not achieving SCI.

Univariate and multivariate analysis of factors related with SCI by day +14 through logistic regression models are depicted in **Table 6**. Significant collinearity was observed between the clinical status assessed by the six-point ordinal scale and the SpO_2/FiO_2 ratio at day 0 (VIF values >6.1). Only the SpO_2/FiO_2 ratio was maintained in the multivariate model since the more objective nature of this variable does not depend on the availability of health care resources (i.e. number of ICU beds). In addition, due to the existence of collinearity between leukocyte and lymphocyte counts and the leukocyte-to-lymphocyte ratio (VIF values >3.5), only the latter parameter was retained.

In the final multivariate model variates inversely related with the probability of achieving SCI included certain comorbidities such as dyslipidemia under statin treatment (odds ratio [OR]: 0.38; 95% CI: 0.19 – 0.73; *P*-value <0.0001) or active solid malignancy (OR: 0.19; 95% CI: 0.04 – 0.94; *P*-value = 0.04) and analytical parameters at day 0 indicating advanced disease such as higher leukocyte-to-lymphocyte ratio (OR [per unitary increment]: 0.94; 95% CI: 0.91 – 0.97; *P*-value = 0.001), higher CRP (OR [per unitary increment]: 0.97; 95% CI: 0.94 – 1.00; *P*-value = 0.065) or LDH levels (OR [per unitary increment]: 0.99; 95% CI: 0.99 – 0.99; *P*-value = 0.013). After adjustment by these factors related with poorer outcomes, TCZ administration within the first 48 hours from admission was still independently associated with a two-fold increase in the probability of SCI by day +14 (OR: 1.98; 95% CI: 1.1 – 3.55; *P*-value = 0.02). Hepatitis expressed by serum ALT levels >100 IU/L also defined a group of patients with a three-fold increased odds of having a favorable response to TCZ therapy (OR: 3.28; 95% CI: 1.3 – 8.1; *P*-value = 0.01).

Although the groups of patients stratified by the timing of TCZ administration

(within the first 48 hours of admission or beyond) were not entirely comparable (**Table S1**), different comorbidity and disease severity variables were also included in the final multivariate model. Early (first 48 hours) initiation of TCZ therapy still retained the statistical significance after such adjustment.

Discussion

A specific analysis of this large series of patients treated with IV TCZ has allowed us to elucidate the factors that may predict a significant clinical response to this immunomodulatory agent in patients with COVID-19. An advantage of this series was the homogeneity in the indications for treatment with TCZ since all patients were selected by a specific committee which applied pre-stablished criteria. All the patients presented bilateral infiltrates on chest X-ray or CT scan, elevated serum CRP levels and respiratory deterioration, overall suggesting hyperinflammatory phase of the disease. Aligned with most of the latest published RCTs (Rosas et al., 2021, Soin et al., 2021, Stone et al., 2020) we chose the clinical response defined as a reduction of at least two scale degrees on clinical status at day 14 after TCZ administration as the primary outcome, as it was deemed to be better explicative of the potential effect of TCZ and less biased that other frequently used variables such as all-cause mortality.

The first interesting finding of the present study was the identification of the early initiation of TCZ therapy as an independent predictor of better clinical response after adjustment by other variables potentially related with the prognosis of severe COVID-19. Patients beginning TCZ therapy within the first 48 hours of admission —as performed in more than half of our cohort—had a two-fold increased probability of presenting a SCI by day +14, after adjustment by other prognostic factors in a multivariate model. Moreover, we observed a gradient in the rates of clinical response according to the time interval between admission and initiation of TCZ (**Figure 1**).

In view of the characteristics of the drug and the specific features of the disease

(McGonagle et al., 2020), early initiation of TCZ in patients with bilateral pneumonia has been generally advised and specifically recommended in most compassionate off-label protocols from the beginning of the pandemics (Mikulska et al., 2020, Moreno Diaz et al., 2021). Although a maximum interval of 48 hours from admission was considered as inclusion criteria in one particular RCT (Salama et al., 2021), no details on the recruitment windows were reported in the majority of published trials (Snow et al., 2021). The impact of the timing of TCZ administration in the clinical response rates had not been accurately analyzed to date. In two previous reports, the potential prognostic benefit of early treatment with TCZ was suggested, although the limited sample included in these studies precluded from performing multivariate analyses to adequately confirm this finding (Martinez-Urbistondo et al., 2021, Moreno Diaz et al., 2021).According to the findings of the present study, a rational conclusion is that patients who reached criteria soon after hospital admission and were in inflammatory phase may benefit more from TCZ administration and we therefore recommend a practical approach work-up including the early detection of patients with COVID-19 bilateral pneumonia fulfilling criteria of hyper inflammatory stage of the disease at the emergency room with daily reevaluation so as to begin TCZ treatment before development of severe ARDS.

Unexpectedly, the finding of hypertransaminasemia (serum ALT levels >100 IU/L) at the time of treatment initiation was also found to act as an independent marker of subsequent response to TCZ. The explanation of this finding is not clear. Efficacy of TCZ is assumed to be more probable when administered in the hyperinflammatory state of COVID-19 (Rodriguez-Bano et al., 2021), and

liver inflammation is considered by some experts as a complication due to immune damage rather than direct viral cytopathic effect (Wu et al., 2021). We postulate that high ALT levels could potentially identify patients at the hyperinflammatory state of the disease. Previous use of statins was related with near a three-fold risk of TCZ failure, which could be explained —in line to what has been shown in a particular study (Mitacchione et al., 2021)— by the potential role of this variable as a subrogate marker of underlying cardiovascular disease, conferring a higher risk of more severe COVID-19 disease.

The profile of patients with favorable response to TCZ therapy in our study was also defined by the absence of major underlying diseases potentially related with a worse outcome, such as active malignancy or the presence of advanced disease with severe respiratory deterioration (as indicated by higher values of CRP or LDH and lower SpO2/FiO2 ratios) (**Table 6**). Such factors have been previously related with poor outcome (Richardson et al., 2020, Wu et al., 2020, Zhou et al., 2020) and efficacy of TCZ has been shown to be lower in advanced stages of SARS-CoV-2-related ARDS (Moiseev et al., 2020). Age was not an independent risk factor for clinical failure in our cohort probably due to the relatively young population included (only one third over 60 years old).

Limitations.

Some limitations of the present research deserve specific consideration. This is a single-center study including patients over the first pandemic wave in Madrid, which entailed overwhelming of health resources and limited access of potentially effective therapeutic alternatives as remdesivir that could have influenced in the late access to hospital care. Therefore, extrapolation to other

centers in different stages of the pandemic should be done with caution. In contrast to the timing from admission, the time interval between symptom onset and TZC administration was not found to have significant influence in clinical response. A possible explanation of this apparent discrepancy could reside on the lower accuracy of the precise calendar date for the initiation of symptoms self-reported by the patient in comparison with the more objective time point expressed by the date of admission. On the other hand, we belief that the beginning of the inflammatory phase of infection is more closely related with the time of symptoms worsening represented by the date of hospital admission. Since stringent criteria were applied to select candidates to TCZ therapy, the present cohort may not be representative of the entire COVID-19 population, particularly elderly patients that were underrepresented in the present cohort. Finally, although the large sample allowed us to perform a robust multivariate model to adequately adjust the main prognostic factors, we cannot rule out the impact of unmeasured confounding factors due to the retrospective nature of the study and the absence of a control group precludes from addressing the potential effect of other administered treatments. In these regard, the heterogeneity in the doses and duration of corticosteroids patients receiving co-administration of corticosteroids that could influence therapeutic outcomes (Khiali and Entezari-Maleki, 2021) , the changes of the treatment guidelines during the study period and the effects of other therapies alongside corticosteroids in each group are other limitations of the study.

Conclusions.

In conclusion, the results of the present study support the early initiation of TCZ therapy in patients with severe COVID-19 and prompt the incorporation of a

recruitment window of 48 hours from admission in future RCTs in order to eventually optimize the efficacy of this treatment.

Appendix

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Conflicts of interest

All the authors declare no potential conflict of interest regarding this study.

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The Clinical Research Ethics Committee approved the study protocol (CEIm no. 20/117) and granted a waiver of informed consent in view of the observational design.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Tables

Table 1. Demographics and baseline characteristics of patients included in the cohort.

Variable	Overall cohort (n = 428)
Age, years [mean ± SD]	55 ± 13.4
Age distribution [n (%)]	
21 to 40 years	57 (13.3)
41 to 60 years	235 (54.9)
61 to 80 years	120 (28)
More than 80 years	16 (3.7)
Male gender [n (%)]	278 (65)
Ethnicity [n (%)][a]	
Caucasian	248 (57.9)
Latino	158 (36.9)
Asian	5 (1.3)
Other	17 (3.9)
Comorbidities [n (%)]	
None	193 (45.1)
Hypertension	136 (31.8)
Dyslipidemia under statin treatment	106 (24.8)
Obesity	74 (17.2)
Diabetes mellitus	72 (16.8)
Atherothrombotic disease	24 (5.6)
Asthma	28 (6.5)
Sleep apnea-hypopnea syndrome	20 (4.7)
Chronic obstructive pulmonary disease	14 (3.3)
Immunosuppression	44 (10.2)
Previous corticosteroid therapy	27 (6.3)
Solid organ transplantation	18 (4.2)
Previous chemotherapy	11 (2.6)
HIV infection	6 (1.4)
Pregnancy	11 (2.6)
Active solid malignancy	17 (4.8)
Active or former smoking	89 (20.7)
Charlson Comorbidity Index [median (IQR)]	1 (0 – 3)
Prior ACEi/ARB therapy [n (%)]	123 (28.7)
Prior anticoagulant therapy [n (%)]	27 (6.3)
Influenza vaccination in the current (2019/20) season [n (%)]	87 (20.3)

ACEi/ARB: angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; HIV: human immunodeficiency virus; IQR: interquartile range; SD: standard deviation. [a] Data on ethnicity not available for 9 patients.

Table 2. Clinical characteristics, laboratory values and radiologic findings at hospital admission.

Variable	Overall cohort (n = 428)
Symptoms at hospital admission [n (%)]	
Cough	317 (74.1)
Dyspnea	309 (72.2)
Fever	209 (48.8)
Myalgia	158 (36.9)
Diarrhea	150 (35)
Vomiting	55 (12.9)
Expectoration	66 (15.4)
Impaired mental status	15 (3.5)
Interval from symptom onset to hospital admission, days [median (IQR)]	7 (5 – 10)
NEWS at hospital admission [median (IQR)][a]	5.5 (3 – 7)
Vital signs at hospital admission	
Axillary temperature, ºC [mean ± SD]	37.8 ± 1.1
Respiratory rate, rpm [median (IQR)]	22 (16 – 30)
Heart rate, bpm [mean ± SD]	101.3 ± 17.3
SpO_2 (at room air) [median (IQR]	92 (88 – 95)
Laboratory values at hospital admission	
Leukocytes, x 10^9 cells/L [mean ± SD]	7.8 ± 3.4
Neutrophils, x 10^9 cells/L [mean ± SD]	6.3 ± 3.3
Lymphocytes, x 10^9 cells/L [mean ± SD]	0.92 ± 0.5
Leukocyte-to-lymphocyte ratio [median (IQR)]	8.5 (5.8 – 13.3)
Platelet count, x 10^9 cells/L [mean ± SD]	237.9 ± 100
ALT, U/L [median (IQR)]	39 (25.3 – 63)
AST, U/L [median (IQR)]	46 (32 – 68)
Creatinine, mg/dL [mean ± SD]	1.03 ± 0.56
CRP, mg/dL [mean ± SD]	16.9 ± 9.2
LDH, U/L [median (IQR)]	426 (356 – 536)
Ferritin, ng/mL [median (IQR)][b]	1,526 (779 – 2,264)
Interleukin-6, pg/mL [median (IQR)][c]	44 (20.2 – 144)
Chest imaging at hospital admission [n (%)]	
Diffuse pneumonia	368 (86)
Multiple lobe pneumonia	26 (6.1)
Single lobe pneumonia	18 (4.2)
No pneumonia	14 (3.3)

ALT: alanine transaminase; AST: aspartate transaminase; bpm: beats per minute; CRP: C-reactive protein; IQR: interquartile range; LDH: lactate dehydrogenase; NEWS: National Early Warning Score; rpm: respirations per minute; SD: standard deviation; SpO_2: pulse oximetry oxygen saturation.

[a] NEWS hospital admission available for 266 patients. [b] Ferritin levels at hospital admission available for 278 patients. [c] Interleukin-6 levels at hospital admission available for 140 patients.

Table 3. Vital signs and laboratory values at day 0, and treatments administered previous to or simultaneously with tocilizumab.

Variable	Overall cohort (n = 428)
Vital signs at day 0	
Axillary temperature, ºC [mean ± SD]	37.4 ± 0.9
Respiratory rate, rpm [median (IQR)]	26 (20 – 30)
Heart rate, bpm [mean ± SD]	88.6 ± 16.4
SpO_2/FiO_2 ratio [median (IQR)]	230 (166 – 321)
Laboratory values at day 0	
Leukocytes, x 10^9 cells/L [mean ± SD]	8.7 ± 5.4
Lymphocytes, x 10^9 cells/L [mean ± SD]	1.04 ± 3.1
Leukocyte-to-lymphocyte ratio [median (IQR)]	10.2 (6.5 – 16.3)
ALT, U/L [median (IQR)]	41 (25 – 65)
AST, U/L [median (IQR)]	43 (31 – 60)
CRP, mg/dL [mean ± SD]	16.3 ± 9.2
LDH, U/L [median (IQR)]	426.5 (356 – 536)
Ferritin, ng/mL [median (IQR)][a]	1,526.5 (779 – 2,264)
Interleukin-6, pg/mL [median (IQR)][b]	53 (17 – 136)
Chest imaging at hospital admission [n (%)]	
Bilateral interstitial infiltrates	217 (50.7)
Bilateral alveolar infiltrates	198 (46.3)
Single lobe infiltrates	7 (1.6)
Other	6 (1.4)
Interval from symptom onset to day 0, days [median (IQR)]	10 (8 – 13)
Interval from hospital admission to day 0, days [median (IQR)]	2 (1 – 4)
Administration of more than one TCZ dose [n (%)]	68 (15.8)
Clinical status according to the six-point ordinal scale at day 0 [median (IQR)]	3 (2 – 3)
Clinical status at day 0 [n (%)]	
2. Hospitalized, no supplemental oxygen requirement	24 (5.6)
3. Hospitalized, low-flow supplemental oxygen requirement (FiO_2 <40%)	188 (44.2)
4. Hospitalized, high-flow supplemental oxygen requirement (FiO_2 ≥40%) or NIMV	190 (44.4)
5. Hospitalized, IMV and/or ECMO	25 (5.8)
Previous or simultaneous therapies [n (%)]	
HCQ	382 (89.2)
LPV/r	154 (35.9)
IFN-β	39 (9.1)
Remdesivir	2 (0.4)
Corticosteroids	218 (51)
Interval to day 0, days [median (IQR)][a]	1 (0 – 2)
Azithromycin	222 (51.8)
Other antibiotics	
Second- or third-generation cephalosporin	289 (67.5)

Amoxicillin/clavulanic acid	100 (23.3)
Carbapenem	14 (3.2)
Fluoroquinolones	15 (3.5)
Others	4 (0.9)

ALT: alanine transaminase; AST: aspartate transaminase; bpm: beats per minute; CRP: C-reactive protein; ECMO: extracorporeal membrane oxygenation; HCQ: hydroxychloroquine; IFN-β: interferon-β; IQR: interquartile range; IMV: invasive mechanical ventilation; LDH: lactate dehydrogenase; LPV/r: lopinavir/ritonavir; NIMV: non-invasive mechanical ventilation; rpm: respirations per minute; SD: standard deviation; SpO_2/FiO_2: SpO_2/FiO_2: pulse oximetry oxygen saturation/fraction of inspired oxygen; TCZ: tocilizumab. [a] Ferritin levels at day 0 available 203 patients. [b] Interleukin-6 levels at day 0 available for 128 patients. [c] Time interval from the initiation of the corresponding therapy to the administration of the first dose of tocilizumab (day 0).

Table 4. Vital signs, laboratory values and clinical status at day 0, 3, 7 and 14 from the initiation of TCZ therapy.

Clinical data	**Day 0**	**Day 3**	**Day 7**	**Day 14**
Main vital signs				
Axillary temperature, ºC [mean ± SD]	37.4 ± 0.9	36.8 ± 0.8	36.8 ± 0.6	36.9 ± 0.5
SpO_2/FiO_2 ratio [median (IQR)]	230 (166 – 321)	268 (170-343)	322 (187-438)	337 (252-448)
Main laboratory values				
Lymphocytes, x 10^9 cells/L [mean ± SD]	1.04 ± 3.1	1.4 ± 3.8	1.5 ± 2.9	1.6 ± 2.2
CRP, mg/dL [mean ± SD]	16.3 ± 9.2	4.2 ± 1.7	1.1 ± 2.9	1.3 ± 3.1
LDH, U/L [median (IQR)]	426.5 (356 – 536)	427(338 – 566)	394(306 – 532)	337(252 – 448)
Clinical status [n (%)]				
1. Discharged to home	0	11 (2.6)	143 (33.4)	256 (59.8)
2. Hospitalized, no supplemental oxygen requirement	24 (5.6)	48 (11.2)	36 (8.4)	21 (4.9)
3. Hospitalized, low-flow supplemental oxygen requirement (FiO_2 <40%)	188 (44.2)	144 (33.6)	71 (16.6)	40 (9.3)
4. Hospitalized, high-flow supplemental oxygen requirement (FiO_2 ≥40%) or NIMV	190 (44.4)	137 (32)	73 (17.1)	32 (7.5)
5. Hospitalized, IMV and/or ECMO	25 (5.8)	69 (16.1)	72 (16.8)	33 (7.7)
6. Death	0	19 (4.4)	33 (7.7)	46 (10.7)
Improvement in clinical status (at least one scale degree) [n (%)]	-	79 (18.5)	207 (48.4)	306 (71.5)
Improvement in clinical status (at least two scale degrees) [n (%)]	-	10 (2.3)	145 (33.9)	271 (63.3)

CRP: C-reactive protein; ECMO: extracorporeal membrane oxygenation; IQR: interquartile range; IMV: invasive mechanical ventilation; LDH: lactate dehydrogenase; NIMV: non-invasive mechanical ventilation; SD: standard deviation; SpO_2/FiO_2: SpO_2/FiO_2: pulse oximetry oxygen saturation/fraction of inspired oxygen.

Table 5. Comparative analysis of factors at the time of the initiation of TCZ therapy (day 0) among patients with or without significant clinical improvement by day +14[a].

Variable	Clinical improvement (n = 271)	No clinical improvement (n = 157)	*P*-value
Age, years [mean ± SD]	52.5 ± 13.1	60.9 ± 12.2	<0.0001
Aged 55 yrs. old or less [n (%)]	271 (64.6)	157 (44.6)	0.0001
Male gender [n (%)]	176 (64.9)	102 (65)	0.661
Non-Caucasian ethnicity [n (%)]	127 (46.8)	53 (33.7)	0.01
Hypertension [n (%)]	71 (26.2)	65 (41.4)	0.001
Dyslipidemia under statin treatment [n (%)]	48 (17.7)	58 (36.9)	<0.0001
Obesity [n (%)]	39 (14.4)	35 (22.3)	0.05
Diabetes mellitus [n (%)]	38 (14)	34 (21.7)	0.05
Atherothrombotic disease [n (%)]	12 (4.4)	12 (7.6)	0.24
Asthma [n (%)]	20 (7.4)	8 (5.1)	0.46
COPD and/or SAHS [n (%)]	13 (4.8)	19 (12.1)	0.01
Immunosuppression [n (%)]	20 (7.4)	24 (15.3)	0.01
Pregnancy [n (%)]	9 (3.3)	2 (1.3)	0.356
Active solid malignancy [n (%)]	4 (1.5)	13 (8.3)	0.001
Active or former smoking [n (%)]	43 (15.9)	46 (29.3)	0.001
Cough at admission [n (%)]	205 (75.6)	112 (71.3)	0.388
Dyspnea at admission [n (%)]	195 (72)	73 (69.5)	0.387
Fever at admission [n (%)]	139 (51.9)	70 (44.6)	0.175
Myalgia at admission [n (%)]	111 (41)	47 (29.9)	0.02
Diarrhea at admission [n (%)]	109 (40.2)	41 (26.1)	0.004
Myalgia and/or diarrhea at admission [n (%)]	172 (63.5)	72 (45.9)	0.0006
Impaired mental status at admission [n (%)]	7 (2.6)	8 (5.1)	0.344
NEWS at admission [median (IQR)]	5 (3 – 7)	6 (4 – 7)	0.144
Diffuse pneumonia at admission [n (%)]	233 (86)	135 (86)	0.854
Axillary temperature at day 0, ºC [mean ± SD]	37.5 ± 1.0	37.5 ± 1.0	0.989
Respiratory rate at day 0, rpm [median (IQR)]	26 (20 – 30)	26 (22 – 30)	0.771
Heart rate at day 0, bpm [mean ± SD]	88.0 ± 17.9	88.2 ± 15.3	0.929
SpO_2/FiO_2 ratio at day 0 [median (IQR)]	288 (181 – 339)	175 (101– 258)	<0.0001
Leukocytes at day 0, x 10^9 cells/L [median (IQR)]	7.3 (5.4 – 10)	7.9 (6.2 – 12.2)	0.007
Lymphocytes at day 0, x 10^9 cells/L [median (IQR)]	0.8 (0.6 – 1.1)	0.6 (0.4 – 0.87)	0.344
Leukocyte-to-lymphocyte ratio at day 0 [median (IQR)]	9.2 (5.8 – 13.3)	12.4 (8.7 – 21.4)	<0.0001
ALT at day 0, IU/L [median (IQR)]	43 (28 – 71)	36 (23 – 58)	0.001
ALT at day 0,>100 IU/L [n (%)]	42 (15.5)	11 (7)	0.008
AST at day 0, IU/L [median (IQR)]	43 (30.5 – 59.5)	44 (31 – 60)	0.888
CRP at day 0, mg/dL [mean ± SD]	15.3 ± 8.9	18 ± 9.45	0.003

LDH at day 0, IU/L [median (IQR)]	409 (327 – 482)	481 (403 – 636.5)	<0.0001
Ferritin at day 0, ng/mL [median (IQR)]	1,479 (808 – 2,115)	1,662 (741 – 2,958)	0.062
Interleukin-6 at day 0, pg/mL [median (IQR)]	53 (16.7 – 116)	55 (18.7 – 251)	0.883
Bilateral alveolar infiltrates at day 0 [n (%)]	112 (41.3)	86 (54.8)	0.009
Interval from symptom onset to day 0, days [mean ± SD]	10.8 (4.6)	11.6 (6.1)	0.13
Interval from admission to day 0, days [mean ± SD]	2.9 (2.6)	4.9 (6)	<0.0001
Tocilizumab in the first two days of admission	271 (60.9)	157 (44.6)	0.001
Clinical status 4 or 5 at day 0 [n (%)]	97 (35.8)	118 (75.2)	<0.0001
Prior or concomitant therapy with remdesivir [n (%)]	8 (3)	8 (5.1)	0.4
Prior or concomitant therapy with HCQ [n (%)]	266 (98.2)	151 (96.2)	0.342
Prior or concomitant therapy with LPV/r [n (%)]	102 (37.6)	73 (46.5)	0.08
Prior or concomitant therapy with IFN-β [n (%)]	16 (9.5)	10 (9.5)	0.988
Prior or concomitant therapy with azithromycin [n (%)]	116 (57.2)	84 (53.5)	0.52
Prior or concomitant corticosteroid therapy [n (%)]	124 (45.8)	94 (59.9)	0.006

ALT: alanine transaminase; AST: aspartate transaminase; bpm: beats per minute; CI: confidence interval; COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein; HCQ: hydroxychloroquine; IFN-β: interferon-β; IQR: interquartile range; LDH: lactate dehydrogenase; LPV/r: lopinavir/ritonavir; NEWS: National Early Warning Score; rpm: respirations per minute; OR: odds ratio; SAHS: sleep apnea-hypopnea syndrome; SD: standard deviation; SpO_2/FiO_2: SpO_2/FiO_2: pulse oximetry oxygen saturation/fraction of inspired oxygen.

[a] Defined by hospital discharge and/or a decrease of ≥2 points from baseline (day 0) on the six-point ordinal scale.

Table 6. Univariate and multivariate analysis of factors related with significant clinical improvement by day +14 from the initiation of TZM therapy.

Variable	Univariate			Multivariate		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Age, years	0.95[a]	0.93 – 0.97	<0.0001			
Non-caucasian ethnicity	1.73	1.15 – 2.6	0.008			
Hypertension	0.5	0.33 – 0.76	0.01			
Dyslipidemia under statin treatment	0.37	0.23 – 0.57	<0.0001	0.38	0.19 – 0.73	<0.0001
Obesity	0.58	0.35– 0.97	0.039			
Diabetes mellitus	0.59	0.35– 0.98	0.043			
COPD and/or SAHS	0.37	0.17– 0.76	0.007			
Immunosuppression	0.44	0.23 – 0.83	0.011			
Active solid malignancy	0.17	0.05 – 0.51	0.002	0.19	0.04 – 0.94	0.04
Active or former smoking	0.45	0.28 – 0.73	0.001			
Myalgia and/or diarrhea at admission	2.05	1.37 – 3.06	<0.0001			
SpO_2/FiO_2 ratio at day 0	1.01[a]	1.00 – 1.01	<0.0001	1.01[a]	1.00 – 1.00	<0.0001
Leukocyte-to-lymphocyte ratio at day 0	0.94[a]	0.91 – 0.96	<0.0001	0.94[a]	0.91 – 0.97	0.001
ALT at day 0 >100 IU/L	2.4	1.2 – 4.9	0.012	3.28	1.3 – 8.1	0.01
CRP at day 0, mg/dL	0.96[a]	0.94 – 0.99	0.004	0.97[a]	0.94 – 1.00	0.065
LDH at day 0, IU/L	0.99[a]	0.99 – 0.99	<0.0001	0.99[a]	0.99 – 0.99	0.013
Bilateral alveolar infiltrates at day 0	0.58	0.39 – 0.86	0.007			
Initiation of TCZ therapy within the first 48 hours from admission	1.93	1.3 – 2.9	0.001	1.98	1.1 – 3.55	0.02
Prior or concomitant corticosteroid therapy	0.56	0.38 – 0.84	0.005			

ALT: alanine transaminase; CI: confidence interval; COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein; LDH: lactate dehydrogenase; OR: odds ratio; SAHS: sleep apnea-hypopnea syndrome; SD: standard deviation; SpO_2/FiO_2: SpO_2/FiO_2: pulse oximetry oxygen saturation/fraction of inspired oxygen.
[a] Odds ratio per unitary increment.

Figure 1. Rates of significant clinical improvement by day +14 according to the timing of TCZ administration.